Journal of Chemical Ecology, Vol. 24, No. 11, 1998

ATTRACTION OF 3-METHYL-1-BUTANOL AND AMMONIA IDENTIFIED FROM *Enterobacter agglomerans* TO *Anastrepha suspensa*

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(Received August 18, 1997; accepted July 14, 1998)

Abstract—Tests demonstrated that volatile chemicals emitted from *Enterobacter agglomerans*, a bacterium that has been isolated from adults as well as fruit infested with larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) and other pest fruit flies, are attractive to female *A. suspensa* in laboratory bioassays. 3-Methyl-1-butanol and ammonia were identified as the two primary volatile chemicals released from active cultures of *E. agglomerans*. No 3-methyl-1-butanol and little ammonia (16.0 μg/hr) are released from sterile tryptic soy agar plates. *E. agglomerans*-inoculated tryptic soy agar plates, however, released an average of 1.5 ± 0.53 μg/hr 3-methyl-1-butanol and 332.9 ± 239.16 μg/hr ammonia after 24 hr of growth. 3-Methyl-1-butanol lures were formulated in a membrane-based system to provide a constant release rate of synthetic chemical. Release rates ranged from 0.046 ± 0.007 to 12.16 ± 2.76 μg/hr. In laboratory tests, equal numbers of females were captured in response to ammonium carbonate lures that released ammonia at the rate of 100 μg/hr and to 3-methyl-1-butanol lures that released 12.16 ± 2.756 μg/hr of synthetic material. The combination of the two lures was more attractive than ammonia alone. Availability of lures formulated for a range of 3-methyl-1-butanol release rates will facilitate field tests of this puta-

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0098-0331/98/1100-1867/$15.00/0

tive microbial attractant and may lead to a better understanding of the role of bacteria in the ecology of pest fruit flies.

Key Words—Insecta, Tephritidae, *Anastrepha suspensa*, *Enterobacter agglomerans*, attractants, volatiles, bacteria, 3-methyl-1-butanol, ammonia, lures.

INTRODUCTION

Bacteria in the family Enterobacteriaceae have been found in association with tephritid fruit flies (e.g., Rubio and McFadden, 1966; Boush et al., 1972; Rossiter et al., 1982; MacCollom and Rutkowski, 1986; Jang and Nishijima, 1990), and bacteria in this family may be strongly attractive to fruit flies (Drew and Lloyd, 1989; Martinez et al., 1994). Bacteria on plant surfaces may serve as a protein source for adult tephritids in nature (Drew et al., 1983). Drew and Fay (1988) hypothesized that increased capture of *Bactrocera tryoni* (Froggatt) in liquid protein bait with bacteria was due to volatile metabolites produced by bacterial growth. Davis et al. (1984) demonstrated that there was greater attraction of Caribbean fruit flies, *Anastrepha suspensa* (Loew), to liquid protein bait solution in McPhail traps versus liquid protein bait placed on cotton wicks in Jackson traps and speculated that this was due in part to volatile end products from microbial breakdown that occurred in the McPhail traps.

Several studies have evaluated the attractiveness of various bacteria found in association with tephritids. Jang and Nishijima (1990) isolated 14 bacterial species from wild and laboratory-reared Oriental fruit flies, *Bactrocera dorsalis* Hendel, and most of these bacteria belonged to the family Enterobacteriaceae. They found that several bacterial species were more attractive to female than to male flies and that washed cells, (cells that were separated from the growth media) were more attractive than water or phosphate buffer. Several strains of *Staphylococcus aureus* were attractive to adults of the Mexican fruit fly, *Anastrepha ludens* (Loew) (Robacker et al., 1991). Attraction to bacterial odors was mediated by feeding history of the flies, as response to bacterial odors decreased with increased sugar hunger (Robacker and Garcia, 1993) and increased with increased protein hunger (Robacker and Moreno, 1995). Ammonia, which is known to be the primary fruit fly attractant that is emitted from liquid protein baits (Bateman and Morton, 1981; Mazor et al., 1987), is produced by microbial growth (e.g., Howell et al., 1988; Scarpati et al., 1996). Several volatile chemicals have been identified from headspace analysis of bacteria and/or culture media (Robacker et al., 1993; Lee et al., 1995; DeMilo et al., 1996), although Robacker and Flath (1995) could not determine if the chemicals that were biologically active in laboratory bioassays were produced by the bacteria or were an artifact of the analytical procedure. Thus, it is not known if there are volatile

chemicals in addition to ammonia that are attractive to fruit flies and produced by actively growing bacteria.

Enterobacter agglomerans is one of several Enterobacteriaceae isolated from adults of the apple maggot, *Rhagoletis pomonella* (Walsh), and from apple maggot-infested fruit (MacCollom et al., 1992). Washed cell preparations of an apple maggot-associated isolate of *E. agglomerans* were attractive to foraging adults in field trials (MacCollom et al., 1992). Culture plates inoculated with *E. agglomerans* are attractive to apple maggot flies in laboratory bioassays (C.R.L., unpublished data). This bacterium has been isolated from adults, larvae, and fruit infested with larvae of the Caribbean fruit fly, *Anastrepha suspensa* (Lowe), field collected in south Florida (C.R.L., unpublished data). Preliminary laboratory studies found that female *A. suspensa* are attracted to volatiles from *E. agglomerans* in laboratory bioassays. Therefore, studies were initiated to quantify ammonia release from *E. agglomerans* washed cells and culture plates and to identify other attractant chemicals released from these substrates. Based on spectroscopic analysis of volatiles, we identified 3-methyl-1-butanol (3-MeBuOH) as a major component. Release rate was quantified, and synthetic 3-MeBuOH was formulated in membrane-based controlled release lures for laboratory tests of biological activity alone and in combination with ammonia.

METHODS AND MATERIALS

E. agglomerans Culture. Stock cultures were maintained in Amherst, Massachusetts. For use in bioassays and chemical analyses, *E. agglomerans* in early log phase were plated on tryptic soy agar (TSA, Difco Laboratories, Detroit, Michigan) and sent via overnight delivery from Amherst, Massachusetts, to Gainesville, Florida. Sterile TSA plates were included as controls. Fresh cultures were shipped periodically to Gainesville and samples of cells subcultured there were shipped back to Amherst for confirmation of culture purity. Upon receipt, plates were left at room temperature overnight to ensure a minimum of 24 hr of growth. Growth of colonies on plates prior to arrival in Gainesville was affected by ambient temperatures during shipment, so there was variation in the amount of growth that occurred before the plates arrived. For comparative purposes, chemical analyses were conducted on plates allowed to grow an additional 24 hr to determine if further growth affected quantitative release of volatile chemicals. After 24 or 48 hr of growth, plates were tested immediately or placed in a refrigerator (7°C) until used. Plates removed from the refrigerator were held at room temperature for at least 1 hr, and plates were used within one week of receipt. For studies that tested washed cells, cells were scraped from the TSA and subcultured in tryptic soy broth (TSB, Difco Laboratories, Detroit, Mich-

igan) at 25°C. *E. agglomerans* was propagated by inoculating one loopful (3 mm diam) of a 24-hr subculture into test tubes containing 10 ml TSB and incubating for 24 hr at 25°C. Cells were separated from the culture medium by centrifugation for 10 min at approximately 17,000*g* (0°C), and the TSB supernatant was discarded (MacCollom et al., 1992). Cells were washed and concentrated by resuspending the pellet in sterile water (deionized), and samples were combined and centrifuged again to remove residual media from cells. This process was repeated twice. The final pellet from 40 ml subculture was resuspended in 10 ml of sterile water to obtain a fourfold increase in concentration.

A. suspensa *Culture.* Caribbean fruit flies used were obtained as pupae from the Florida Department of Agriculture and Consumer Services, Division of Plant Industry in Gainesville. Flies were maintained as reported previously (Epsky et al., 1993). Females that were 4–12 days posteclosion were used for all trials. Fully fed flies were used for all bioassays to reduce biased attraction to ammonia, which is enhanced by subjecting flies to protein starvation prior to testing (Robacker, 1991).

Volatile Collection and Chemical Analysis. Capillary gas chromatography (CGC) analyses were conducted to identify and quantify volatile chemicals from *E. agglomerans*. Headspace volatiles were collected for 1 hr at 1 liter/min airflow using purified air and were analyzed by collection systems described previously (Heath and Manukian, 1992; Heath et al., 1993). Initially, volatile collections were made from washed cells (20 ml), TSB supernatant (20 ml), and sterile TSB (40 ml). The amount of volatile chemicals released from washed cells was too low to analyze by CGC with standard on-column injection techniques. Therefore, volatiles were introduced into the CGC by thermal desorption injection. Volatiles were collected in 6-mm-OD by 120-mm-long Pyrex tubes packed with 15-mm bed length of adsorbent (Tenax TA 60/80 mesh) and were thermally desorbed in a Tekmar cryofocusing automatic desorber onto a 30-m by 0.25-μm methyl silicone capillary column interfaced to a Finnigan ion trap detection system mass spectrometer. Volatiles were analyzed by electron impact (EI) and by chemical ionization (CI) with isobutane as the reagent gas. Standard on-column injection techniques were used for analyses of sterile and *E. agglomerans*-inoculated TSA plates in a Hewlett-Packard model 5890A Series II gas chromatograph, equipped with a cool on-column capillary injector (septum injector) and flame ionization detector. Mass spectra were obtained from capillary columns, operated as described above, coupled to a Finnigan ITDS mass spectrometer in either EI or CI mode. The reagent gas used for chemical ionization was isobutane.

The release rates of ammonia from washed cell preparations, sterile, and *E. agglomerans*-inoculated TSA plates were determined with an ammonia-specific ion-selective electrochemical probe (Orion, Boston, Massachusetts). Test substrates were placed into wide-mouth Erlenmeyer flasks (500 ml), and 13 ml

of washed cells were added to 87 ml tap water for testing. TSA plates were cut into four sections to facilitate placement into the flask. Sections were removed individually from a Petri dish and placed inoculated side up around the bottom of the flask so that all sections were exposed to airflow. The flask was purged for 1 hr with an airflow of 1 liter/min, and volatiles were directed to a sparge system that consisted of a gas dispersion tube (No. 7198 Ace Glass, Vineland, New Jersey) placed in a graduated cylinder containing 100 ml of HCl solution (0.05 N). After a collection, the ionic strength of the sample solution was adjusted with 5 M NaOH/0.05 M disodium EDTA/10% methanol containing a color pH indicator. A standard ammonium calibration curve was prepared each day an analysis was done.

Formulation of Synthetic Chemicals. Ammonium carbonate was formulated to release ~100 μg/hr ammonia by packing approximately 20 mg into the bottom of a heat-sealed 200-μl glass capillary pipette (Becton, Dickinson and Co., Parsippany, New Jersey). The 3-MeBuOH was formulated by using a membrane-based formulation system described previously (Heath et al., 1996). Briefly, a lure (3 × 5 cm) with a 1.17-cm-diam. hole in the center of the front was prepared from 6-mil impermeable polyethylene. Release rate was governed by using 1-mil high-density polyethylene (membrane) film (Consep Inc., Bend, Oregon) that was placed inside the lure. The release area of the membrane was reduced to a 3-, 5-, or 10-mm-diam. circle by placing a piece of aluminum tape (United Tape Company, Cumming, Georgia) over the 1.17-cm hole in the lure. Lures contained filter paper and a plastic grid to provide mechanical stability. They were loaded with 5 or 10 μl (low dose) or 50 μl (high dose) of 3-MeBuOH and were placed in a hood with a 0.25 cm/sec airflow for 24 hr before use in a laboratory bioassay. Longevity was determined for lures loaded with 50 μl of 3-MeBuOH and with the 3-, 5- and 10-mm membrane release area. Release rates from two lures of each membrane release area were measured after four days and then every three to four days over a period of 21 days by using the same methods as those used for TSA plates. Lures were kept in a hood between measurements at ambient temperature and with an airflow of ~0.2 m/sec. Differences in release rates over time were compared with a heterogeneity of slopes model by using Proc GLM (SAS Institute, 1985). Mean release rates were used in linear regression analysis to determine the change in rate over time and the half-life of each lure.

Laboratory Bioassay of A. suspensa. All bioassays were conducted as two-choice bioassays by using 30.2- × 30.2- × 122-cm flight tunnels (Heath et al., 1993; Epsky et al., 1993). Tests were run in a greenhouse under natural light conditions. Liquid test substrates were placed in 500-ml narrow-mouth flasks. Solid test substrates were placed in 1.9-liter wide-mouth plastic jars (Anchor Hocking, St. Paul, Minnesota). Test substrates were vented into the tunnels for at least 1 hr before the addition of flies to stabilize the release of volatiles. New

plastic jars were used for each change in test substrate, unless a higher concentration of the same substrate was used in subsequent bioassays. Two horizontally mounted traps (140-ml clear plastic vial, BioQuip, Gardena, California) were suspended upwind inside the tunnel (Heath et al., 1993). A piece of fluorescent orange adhesive paper (6.5 × 6.5 cm; Atlantic Paste and Glue Co., Brooklyn, New York), was attached to the outside face of the trap with double-sided tape. This provided a visual cue (Greany et al., 1977; Sivinski, 1990) and a sticky surface to capture responding flies. Test substrate volatiles were introduced into the tunnel through the trap, and the trap face and the adhesive paper had 1.5-cm-diam. holes in the center to allow point source release of test substrate volatiles. The positions of the two test substrates were switched after each test to reduce position effects. Twenty females were released at the downwind end of the tunnel, and the number of flies captured on the adhesive paper trap face was recorded after approximately 20 hr. Flies were given water, but no food, during a bioassay.

In the first experiment, 1.7 and 17 ml of washed cells in 99.3 and 83 ml of tap water, respectively, were tested against a blank (100 ml of tap water). Washed cells were added to water to prevent desiccation during the bioassay. The experiment was replicated eight times. The second experiment was conducted to confirm the biological activity of 3-MeBuOH and to determine the concentration of 3-MeBuOH emitted from the washed cell preparation. Females were given the choice of 13 ml of washed cells in 87 ml of tap water and a low dose 3-MeBuOH lure at one of three release rates (lure with 3-mm membrane release area and loaded with 5 μl of 3-MeBuOH and one or two lures with 3-mm membrane release area and loaded with 10 μl of 3-MeBuOH). Each comparison was replicated six times. In subsequent testing, flies were given the choice of volatiles from *E. agglomerans*-inoculated and sterile TSA plates. Initial tests confirmed that volatiles from *E. agglomerans*-inoculated TSA plates captured more females than uninoculated plates. However, there was a large amount of variation in chemical release among the inoculated TSA plates and the inoculated TSA plates released a large amount of ammonia, which is known to be attractive to fruit flies, in comparison to sterile TSA plates tests (see Results). Therefore, the third experiment used synthetic lures in place of *E. agglomerans*-inoculated plates to standardize release rates and was designed to compare attraction due to ammonia with attraction due to 3-MeBuOH. Females were given the choice of an ammonia lure or a 3-MeBuOH lure loaded with 100 μl of 3-MeBuOH with either a 3-, 5- or 10-mm membrane release area. The test was replicated four times. The fourth experiment was conducted to determine if the combination of ammonia and 3-MeBuOH was more attractive than ammonia alone. The test was conducted five times and used the same lure formulations as experiment 3. Two-sample t tests (Proc TTEST; SAS Institute, 1985) were used for comparisons between the two choices offered together.

RESULTS

Chemical Analysis of E. agglomerans *Volatiles.* Analysis of volatiles from *E. agglomerans* found a single major peak with a retention time of 6.5 min in both washed cells and *E. agglomerans*-inoculated TSA plates. Integration of chemicals detected showed that this peak accounted for >85% of total chemicals detected. A library search (National Institute of Standards and Technology Library, Gaithersburg, Maryland) based on EI mass spectral data provided a significant match of the unknown with 3-MeBuOH. Confirmation was provided by comparison with synthetic 3-MeBuOH (Aldrich, St. Louis, Missouri). Chemical ionization mass spectra of natural and synthetic 3-MeBuOH were identical. This peak was also found in volatiles from the TSB supernatant from *E. agglomerans* culture; however, it was one of numerous peaks and was not the major peak. No 3-MeBuOH was detected among volatiles from sterile TSB media.

No ammonia was detected from washed cell preparations. The amount of 3-MeBuOH from these was variable, and the amounts observed ranged from 50 to 200 pg/hr ($N = 5$). Because of the low amounts observed, no attempt was made to quantify release of 3-MeBuOH from washed cells. Chemical analysis indicated little ammonia (16.0 μg/hr, $N = 2$) and no 3-MeBuOH released from sterile TSA plates. *E. agglomerans*-inoculated TSA plates, which contained actively growing colonies of bacteria, released 332.9 μg/hr ($N = 10$) ammonia and 1.48 μg/hr ($N = 4$) 3-MeBuOH after 24 hr of growth. There was large variability in release rates of both chemicals among inoculated plates, as ammonia and 3-MeBuOH release rates ranged from 54.8 to 684.4 and 0.80 to 2.28 μg/hr, respectively. Ammonia and 3-MeBuOH release rates from plates after 48 hr of growth averaged 895.0 and 2.48 μg/hr, respectively. No attempts were made to quantify bacterial growth on the TSA plates before chemical analysis, but variation in amount of growth that occurred before arrival in Gainesville apparently contributed to variation in release rates obtained from the inoculated plates.

Release Rates from 3-MeBuOH Lures. Initial release rates (mean ± SD) from the low-dose 3-MeBuOH lures (i.e., with 3-mm membrane release areas) were 0.05 ± 0.007, 0.08 ± 0.018, and 0.19 ± 0.018 μg/hr for 5-μl, 10-μl, and for two 10-μl-loaded lures, respectively. These lures were used in the laboratory bioassay comparisons with washed cells. Initial release rates from the high-dose lures (i.e., loaded with 50 μl) were 1.23 ± 0.30, 5.44 ± 0.78, and 12.16 ± 2.76 μg/hr for lures with 3-, 5- and 10-mm-diam. membrane release areas, respectively. There were significant differences in both the y intercepts ($F = 156.14$; $df = 2, 42$; $P = 0.0001$) and the slopes of the regressions ($F = 31.55$; $df = 2, 42$; $P = 0.0001$) of 3-MeBuOH release (y = μg/hr) versus days (x) for each lure type (Figure 1). Release rate decreased in all lures over time ($\beta_1 = -0.0429$, $r^2 = 0.96$; $\beta_1 = -0.19367$, $r^2 = 0.98$; and $\beta_1 = -0.34460$,

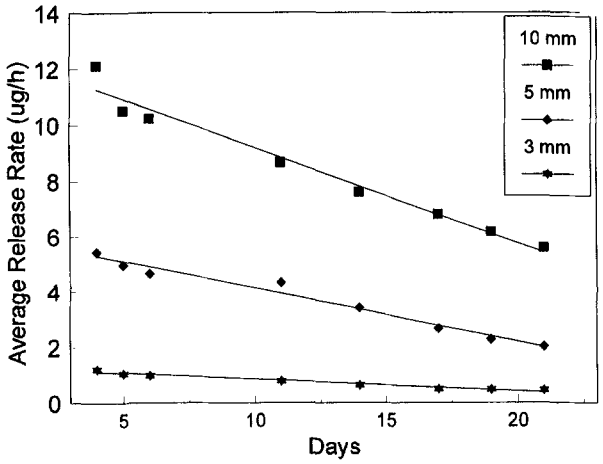

FIG. 1. Average release rates over time of 3-methyl-1-butanol formulated in membrane-based lures. Regressions were determined from lures (N = 2) containing 50 μl of 3-methyl-1-butanol and with membrane release areas of 3 (solid line, star), 5 (solid line, diamond), and 10 mm (solid line, square).

r^2 = 0.97 for 3-, 5- and 10-mm membrane release areas). Generally the half-life of the lures was 16 days.

Laboratory Bioassay of A. suspensa. More flies were captured in response to 17 ml of washed cells than to a water blank, although there was no difference when 1.7 ml of washed cells were tested (Table 1). The 3-MeBuOH lures that

TABLE 1. RESULTS OF TWO-CHOICE BIOASSAY TESTS: EXPERIMENTS 1 AND 2[a]

Choice 1		Choice 2				
Treatment	Response	Treatment	Response	t	df	P
Experiment 1						
1.7 ml cells	4.6 ± 3.58	water control	4.7 ± 1.67	0.0894	14	ns[b]
17 ml cells	8.1 ± 3.09	water control	2.0 ± 1.51	5.0349	14	0.0002
Experiment 2						
13 ml cells	4.8 ± 1.83	0.046 μg/hr	1.3 ± 1.21	3.8996	10	0.0030
13 ml cells	5.8 ± 2.92	0.082 μg/hr	2.2 ± 5.83	2.0117	10	0.0720
13 ml cells	6.7 ± 3.50	0.187 μg/hr	3.3 ± 3.56	1.6352	10	ns

[a] Response (mean ± SD) of 20 female *A. suspensa* per test to volatiles from washed *E. Agglomerans* cells versus a control (experiment 1) or several concentrations of 3-methyl-1-butanol synthetic lures (experiment 2).
[b] Not significant.

TABLE 2. RESULTS OF TWO-CHOICE BIOASSAY TESTS: EXPERIMENT 3[a]

Choice 1		Choice 2				
Treatment	Response	Treatment	Response	*t*	*df*	*P*
1.23 μg/hr lure	5.0 ± 2.16	ammonia	11.5 ± 3.70	3.0361	6	0.0229
5.44 μg/hr lure	5.3 ± 1.70	ammonia	12.3 ± 4.19	3.0921	6	0.0213
12.16 μg/hr lure	9.0 ± 2.16	ammonia	9.3 ± 3.86	0.1130	6	ns[b]

[a] Response (mean ± SD) of 20 female *A. suspensa* per test to volatiles from several concentrations of 3-methyl-1-butanol synthetic lures (average release rate) versus ammonia (100 μg/hr).
[b] Not significant.

released 0.187 μg/hr captured as many flies as washed cells (Table 1), confirming the biological activity of 3-MeBuOH and indicating the approximate release rate from this concentration of washed cells. Overall capture of flies in these tests, however, was low and only half the flies were captured in any of the bioassays. Therefore, tests were continued with higher release rates of 3-MeBuOH, and lures were formulated to mimic release from an *E. agglomerans*-inoculated TSA plate at the lowest concentration (i.e., lure with 3-mm membrane release area loaded with 50 μl 3-MeBuOH), and at 5- and 10-fold higher concentrations (i.e., lures with 5- and 10-mm membrane release areas, respectively, loaded with 50 μl 3-MeBuOH). In experiment 3, the three concentrations of 3-MeBuOH were tested against 100 μg/hr of ammonia, which is an optimal concentration for tests in our bioassay system (N.D.E., R.R.H., and B.D.D., unpublished data). The 3-MeBuOH at the two lower release rates captured fewer females than the ammonia, but 3-MeBuOH, at the highest release rate tested, captured as many flies as ammonia (Table 2). When tested in combination with ammonia in experiment 4, the highest concentration of 3-MeBuOH combined with ammonia captured more females than the ammonia alone (Table 3). In bioassays in which both ammonia and the highest dose 3-MeBuOH lure were offered, either alone (experiment 3) or in combination (experiment 4), an average of 18 of 20 flies in the bioassay were captured.

DISCUSSION

3-Methyl-1-butanol has been identified previously from fruit-fly-attractive substances. For example, 3-MeBuOH was one of 28 chemicals emitted from fermented host fruit of *A. ludens* (Robacker et al., 1990). It was not attractive by itself and was removed from further consideration as a host fruit attractant. A literature search indicated that 3-MeBuOH was the major volatile obtained from autoclaved supernatant of 8-day-old cultures of *Klebsiella pneumoniae*-

TABLE 3. RESULTS OF TWO-CHOICE BIOASSAY TESTS: EXPERIMENT 4[a]

Choice 1		Choice 2				
Treatment	Response	Treatment	Response	*t*	*df*	*P*
1.23 μg/hr lure + ammonia	8.6 ± 4.15	ammonia	9.2 ± 2.17	0.2860	8	ns[b]
5.44 μg/hr lure + ammonia	8.6 ± 4.03	ammonia	8.8 ± 1.30	0.1054	8	ns
12.16 μg/hr lure + ammonia	11.6 ± 2.41	ammonia	7.0 ± 3.31	2.5095	8	0.0364

[a] Response (mean ± SD) of 20 female *A. suspensa* per test to volatiles from several concentrations (average release rate) of 3-methyl-1-butanol synthetic lures plus ammonia versus ammonia alone. A 100 μg/hr ammonia release rate was used in all tests.
[b] Not significant.

inoculated TSB (Lee et al., 1995) and of 4- and 8-day-old cultures of *Citrobacter freundii*-inoculated TSB (DeMilo et al., 1996). There were 20 and 21 chemical components, respectively, identified in addition to this compound. However, there was no information regarding the attractiveness of the individual chemicals. In comparisons of autoclaved supernatant to nonautoclaved supernatant of 4-day-old *C. freundii*-inoculated TSB, the amount of 3-MeBuOH was greatly reduced in the autoclaved supernatant with no corresponding change in *A. ludens* attraction in laboratory bioassays (DeMilo et al., 1996). 3-MeBuOH was identified as a minor component in vacuum steam distillation extraction of corn protein hydrolyzate bait (Buttery et al., 1983).

3-MeBuOH production may be limited to certain species, as no 3-MeBuOH was identified among volatiles from 6-day-old cultures of *Staphylococcus* species-inoculated TSB (Robacker et al., 1993). MacCollom et al. (1992) found that more apple maggot flies were captured on traps baited with washed cells of *E. agglomerans* than with washed cells of *Klebsiella oxytoca*, *Enterobacter cloacae*, *Pseudomonas fluorescens*, or *Bacillus cereus*. It is not known if these species produce 3-MeBuOH. Variation in fruit fly attraction also may occur within species. *E. agglomerans* isolates from different sources varied in their ability to attract apple maggot flies and that attraction may be related to the substrates on which the isolates are growing (C.R.L., unpublished). Further studies are needed to determine if production of 3-MeBuOH is limited to particular bacterial species, specific bacterial isolates, and/or bacteria growing on certain substrates, and if attraction to bacteria is correlated with or enhanced by production of 3-MeBuOH.

The role of bacteria in the ecology of tephritid fruit flies is poorly under-

stood (reviewed in Drew and Lloyd, 1989). Several studies have noted that, among all the bacteria that fruit flies encounter during feeding, only a few are repeatedly recovered internally from fruit flies or their food. Vijaysegaran et al. (1997) found that structures on the mouthparts of *Bactrocera* species limited the size of bacteria that could be ingested by these flies. Thus, since only a subset of bacteria present in the environment could serve as a food source, they speculated that flies may be selectively attracted to fruit or leaf surfaces on which these bacterial species are growing. Attractiveness of fruit fly-associated *E. agglomerans* isolates was correlated with the presence of uricase, an enzyme that degrades uric acid, and *E. agglomerans* isolates that produce uricase constitutively have been isolated from bird dung (C.R.L., unpublished). Thus, bacteria in bird dung could degrade uric acid, which is excreted by birds, and make bird dung a more suitable nitrogen source for foraging. Adults of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the apple maggot feed on bird dung in the field (Hendrichs and Hendrichs, 1990; Hendrichs and Prokopy, 1990). Bird dung was attractive to adults of both species (Prokopy et al., 1992, 1993) and to adults of *A. suspensa* (Epsky et al., 1997). Thus, fruit flies attracted to bird dung on which an appropriate *E. agglomerans* isolate is growing may obtain protein by direct consumption of the bacteria themselves, as well as by feeding on substrate altered by microbial degradation.

Both ammonia and 3-MeBuOH were needed for optimal *A. suspensa* attraction in our studies. Synthetic 3-MeBuOH alone may be more attractive to apple maggot flies than to *A. suspensa*, because small amounts of washed cells (10 μl) enhanced capture of apple maggot flies in field trials (MacCollom et al., 1992), but 13 ml of a fourfold concentration of washed cells was needed for low-level attraction of *A. suspensa* in laboratory trials reported here. Release of ammonia alone may be responsible for *A. suspensa* attraction to *E. agglomerans*-inoculated TSA plates, because small amounts of 3-MeBuOH were released relative to ammonia. However, TSA represents an artificial substrate, and additional studies are needed to determine the amount of 3-MeBuOH released relative to ammonia from *E. agglomerans* growing on natural substrates.

Field studies of traps baited with 3-MeBuOH alone and in combination with ammonia and other previously identified fruit fly attractants, such as putrescine, acetic acid (Heath et al., 1995), and trimethylamine (Heath et al., 1997), are needed to test the effectiveness of these materials for use in traps for pest fruit flies. Such studies may lead to a better understanding of the role of bacteria in the ecology of fruit flies. In field tests of combinations of ammonia, acetic acid, putrescine, and trimethylamine, capture of other pest insects was observed, e.g., Lepidoptera and Blattodea. Availability of lures that can be formulated for a range of 3-MeBuOH release rates will facilitate field tests of various combinations of these synthetic attractants for pest fruit flies as well as for a variety of other pest insects.

Acknowledgments—We thank Marjorie Nguyen, Yasmin Cardoza, Lushantha Gunasekera, and Rodrigo Cabezas (University of Florida, Gainesville, Florida) for technical assistance; Michael Bentivegna, Jr., and the Atlantic Paste and Glue Co., Inc. (Brooklyn, New York), for providing the adhesive paper; Janice Gillespie, Edie Christensen, and Consep Membranes, Inc. (Bend, Oregon) for providing materials for lures; and A. B. DeMilo (USDA/ARS, Beltsville, Maryland), S. Opp (California State University, Hayward), D. Moreno (USDA/ARS, Weslaco, Texas), J. J. Duan (University of Hawaii), and R. Sjogren (Department of Microbiology, University of Vermont) for reviewing earlier versions of this manuscript. This research was funded in part by the California Department of Food and Agriculture (93-0478) and by the Office of Exploratory Research-United States Environmental Protection Agency (Project R819490-0). This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the USDA.

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